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Authors

Takemura, R
Stenberg, PE
Bainton, DF
et al.

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Rapid Redistribution of Clathrin onto Macrophage Plasma Membranes in Response to Fc Receptor-Ligand Interaction During Frustrated Phagocytosis

Reiko Takemura, Paula E. Stenberg, Dorothy F. Bainton, and Zena Werb

Laboratory of Radiobiology and Environmental Health, and the Departments of Anatomy and Pathology, University of California, San Francisco, California 94143. Dr. Takemura's present address is Department of Anatomy, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan. Address reprint requests to Zena Werb.

Abstract. We have observed increases in assembled clathrin on the plasma membrane during "frustrated phagocytosis," the spreading of macrophages on immobilized immune complexes. Resident macrophages freshly harvested from the peritoneal cavity of mice and attached to bovine serum albumin (BSA)-anti-BSA-coated surfaces at 4°C had almost no clathrin basketworks on their adherent plasma membrane (<0.01 coated patch/μm^2), as observed by immunofluorescence, immunoperoxidase, and platinum-carbon replica techniques, although abundant assembled clathrin was observed in the perinuclear Golgi region. When the cells were warmed to 37°C they started to spread by 4 min and reached their maximum extent by 20 min. Spreading preceded clathrin assembly at the plasma membrane. Clathrin-coated patches were first observed on the adherent plasma membrane at 6 min. Between 12 and 20 min assembled clathrin coats appeared on both adherent and nonadherent plasma membranes with a concomitant decrease in identifiable clathrin in the perinuclear region. A new steady state emerged by 2 h, as perinuclear clathrin began to reappear. At 20 min at 37°C the adherent plasma membranes of macrophages spreading on BSA alone had 0.9 coated patch/μm^2, whereas in cells spread on immune complex–coated surfaces, the clathrin patches increased, dependent on ligand concentration, to a maximum of 2.1 coated patches/μm^2. Because frustrated phagocytosis of immune complex–coated surfaces at 37°C increased the area of adherent plasma membrane, the total area coated by clathrin basketworks increased 5-fold (28 μm^2/cell) as compared with cells plated on BSA alone (5.6 μm^2/cell) and 200-fold as compared with cells adhering to immune complexes at 4°C.

We then determined that macrophages cultured on BSA-coated coverslips for 24 h already have abundant surface clathrin. When immune complexes were formed by the addition of anti-BSA IgG to already spread macrophages cultured on BSA-coated coverslips for 24 h, clathrin assembled at the sites of ligand-receptor interaction even at 4°C, before spreading, and a 2.6-fold increase in assembled clathrin was observed on the adherent plasma membrane of cells on immune complexes as compared with cells on BSA alone. Clathrin was reversibly redistributed to the Golgi region, returning to the steady state by 2 h. These data indicate that receptor-ligand interaction during frustrated phagocytosis induces clathrin assembly on the plasma membrane and reversibly diminishes the assembly of clathrin in the perinuclear Golgi region, and suggest that assembled clathrin may play a role in the dynamic changes in membrane distribution in these cells.

CLATHRIN-COATED pits and vesicles are involved in receptor-mediated endocytosis (1, 5), but little is known about the factors that influence recruitment of clathrin at the cell surface. Although the total number of coated pits has been reported to be the same before and after ligands are given to cells (58), capping of surface IgG on lymphocytes induces an increase in surface-associated coated pits (49), and clathrin-coated pits appear at the cell surface in increased amounts during the development of mosquito oocytes to the vitellogenic stage (46, 47). A cycle of assembly and disassembly of clathrin basketworks probably is involved in endocytosis (23). A significant pool of soluble or unassembled clathrin triskelions (20, 25, 31, 46), which may serve as intermediates in assembly and disassembly, has been demonstrated in cells. In addition to the endocytic clathrin-coated pits and vesicles near the surface of the cells, clathrin-coated vesicles are observed within cells near Golgi complexes, where they are probably involved in the transport of membranes and contents vectorially through the Golgi complexes and secretion vacuoles (7, 16, 48).

High-resolution electron microscopy has been used to identify large clathrin-coated patches on phagosomes in macrophages that have ingested latex beads (1), and clathrin-coated patches have also been observed at sites of interactions of macrophages with opsonized erythrocytes (3, 36). These observations have suggested that clathrin assembly may be triggered by receptor-ligand interactions in macrophages. The spreading of macrophages on immune complex–coated surfaces is believed to represent an attempt to phagocytose these surfaces and has been used to analyze membrane receptor mobilization (21, 32, 60). This model system, called frustrated phagocytosis (24), is suitable for studying clathrin assembly because a large area of plasma membrane surface that interacts with the ligand is fixed on the ligand-coated surface and is thus available for examination. In the present study we have determined that clathrin assembly at the cell surface is induced by receptor-ligand interaction in this model system of Fc receptor–mediated phagocytosis and occurs with a concomitant decrease in clathrin-coated structures in the Golgi region.

Materials and Methods

Macrophages

Resident peritoneal macrophages were obtained from female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) by lavaging the peritoneum with phosphate-buffered saline (PBS) containing 50 U/ml heparin (54).

Antibodies

An IgG fraction of rabbit anti–bovine serum albumin (BSA) antibody, rhodamine-conjugated IgG fraction of sheep anti–rabbit IgG, and rhodamine-conjugated IgG fraction of affinity-purified goat anti–rabbit IgG were obtained from Cappel Laboratories (West Chester, PA). Mouse anti-BSA antibody was raised by injecting BSA in Freund's complete adjuvant into CD-1 mice and then by boosting twice with BSA alone. The IgG fraction was purified from the serum by means of protein A-Sepharose (13). Affinity-purified rabbit anticlathrin antibody, a gift of Dr. Daniel Louvard (Pasteur Institute, Paris) has been described previously (26). This polyclonal anticlathrin antibody, which was raised against empty clathrin cages prepared from pig brain coated vesicles by extraction with 2 M urea, recognizes both heavy and light chains of clathrin from various species, as tested by Western blotting (26).

Immune Complex–coated Coverslips

Coverslips coated with immune complexes were prepared by the procedure of Michl et al. (33) using 3 mg/ml BSA and rabbit or mouse anti-BSA IgG at various concentrations. Glass coverslips were treated with poly-L-lysine (M_r 150,000–350,000, Sigma Chemical Co., St. Louis, MO) and then with glutaraldehyde before binding with BSA and anti-BSA IgG. For all immunocytochemistry experiments, macrophages were spread on coverslips on which immune complexes were formed with BSA and 0.12 mg/ml IgG from a mouse anti-BSA antiserum, a concentration shown to induce maximum spreading.

The immobilized immune complexes used in these experiments were in an apparently homogeneous layer; they were uniform as observed by light microscopy with fluorescein-labeled anti–BSA IgG and by thin-section transmission electron microscopy using a peroxidase-labeled second antibody (sheep Fab anti–rabbit IgG) reactive against rabbit anti–BSA IgG (52). Up to 2.5 h macrophages did not appear to ingest or degrade immune complexes. This was tested by using immune complex–coated surfaces formed by fluorescein-labeled anti-BSA IgG. If macrophages had taken up or degraded the immune complexes, darkened spots would have appeared in the bright carpet, as has been observed with cells plated on fibronectin-coated surfaces (6, 9). Occasional clearing of small areas was observed, but this was always mediated by polymorphonuclear leukocytes, which contaminate these preparations at a low concentration (<1%).

Determination of IgG Density on Immune Complex–coated Coverslips

Coverslips coated with BSA were incubated with fluorescein-conjugated rabbit anti-BSA (IgG fraction or $F(ab')_2$ fragments). After the unbound IgG or $F(ab')_2$ was washed away, the bound conjugate was eluted in 0.01 M NH_4OH and fluorescence was measured by excitation of 495 nm and emission of 519 nm. The amount of anti–BSA IgG was calculated by using a standard curve of known concentrations of anti–BSA IgG in the same pH buffer.

Spreading of Macrophages on Immune Complex–coated Coverslips

Freshly harvested macrophages were attached to and spread onto preformed immobilized BSA-anti-BSA immune complexes by a procedure modified from Rabinovitch and DeStefano (45). For precise temperature control, an aluminum cooling block (32) that allowed a rapid switch from 4 to 37°C was used. After the switch from 4°C to warm water, 34°C was attained by 1 min. The temperature of the block was monitored by a surface temperature probe connected to a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH), and the temperatures were maintained at ± 0.1°C. BSA-anti-BSA-coated coverslips were placed on the cooling block, which had been precooled to 4°C. Macrophages suspended in ice-cold Eagle's minimal essential medium supplemented with 25 mM Hepes buffer, pH 7.4, were overlaid onto coverslips ($1–10 \times 10^5$ cells/cm^2) and allowed to attach for 60 min at 4°C. The coverslip cultures were washed free of nonadherent peritoneal cells and covered with a drop of ice-cold Eagle's minimum essential medium–Hepes. Then the coverslips were warmed to 37°C by changing from the circulating water bath that perfused the block to the warm circulating water bath. For incubations longer than 20 min, the coverslips were transferred into medium in a multiwell plate and cultured at 37°C in a humid 5% CO_2 incubator. In some experiments cells were attached to the BSA-anti-BSA-coated coverslips at room temperature for 5–10 min.

A second method for formation of immune complexes, called the underlay method, was used for macrophages first cultured on BSA alone for 24 h. Anti-BSA IgG was then added to form immune complexes, and cells were allowed to spread according to the procedure of Michl et al. (32, 33). Macrophages suspended in Dulbecco's modified Eagle's medium supplemented with penicillin, streptomycin, and 0.2% lactalbumin hydrolysate were plated onto BSA-coated coverslips in a multiwell plate ($1–2.5 \times 10^5$ cells/cm^2) and incubated for 2 h at 37°C in a humid 5% CO_2 incubator before being washed free of nonadherent peritoneal cells and cultured overnight in Dulbecco's modified Eagle's medium–lactalbumin hydrolysate. The cells cultured overnight on BSA were washed with warm Dulbecco's modified Eagle's medium–lactalbumin hydrolysate and placed onto the cooling block at 4 ± 0.1°C. The anti-BSA IgG was added and the coverslips were incubated for 60 min at 4°C on the cooling block. Anti-BSA IgG penetrated under most parts of the cells and formed a uniform immune complex, as shown by the use of fluorescein-labeled anti-BSA IgG. After the excess soluble anti-BSA IgG was removed, the coverslips were returned to the cooling block and covered with Eagle's minimum essential medium-Hepes, and the cooling block was warmed to 37°C. For incubations longer than 20 min, the coverslips were transferred to a CO_2 incubator at 37°C.

Indirect Immunofluorescence

Macrophages were fixed in 2% paraformaldehyde in PBS for 10–20 min at ambient temperature. Cells were then rinsed with PBS twice, incubated for 10 min in PBS containing 0.1 M glycine or 0.05 M NH_4Cl to quench any remaining aldehyde reactivity, and rinsed again in PBS. The fixed cells were then rendered permeable to protein reagents by 10-min exposure to 0.1% Triton X-100 in PBS. The cells were washed in PBS containing 3% normal sheep serum, and coverslips were overlaid with rabbit anticlathrin antibody diluted 1:200 in PBS containing 3% normal sheep serum. After a 60–90-min incubation at ambient temperature, the coverslips were washed thoroughly with PBS or with PBS that contained 3% normal sheep serum. Samples were then incubated for 30–60 min at ambient temperature with rhodamine-conjugated IgG fraction of sheep anti–rabbit IgG or rhodamine-conjugated affinity-purified goat anti–rabbit IgG diluted 1:100. The coverslips were thoroughly washed in PBS and then observed with a Zeiss Photomicroscope III equipped for epi-illumination.

Immunoperoxidase Procedures

The procedures used were essentially the same as those described previously (51). Macrophages were fixed in 2% paraformaldehyde and 0.05% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 h at ambient temperature. After incubation at ambient temperature for 90 min with rabbit anticlathrin antibody or control substance, the cells were washed six times and then incubated for 90 min at ambient temperature in Fab fragments of sheep anti–rabbit IgG conjugated to peroxidase (Pasteur Institute) at a dilution of 1:50. The cells were washed and then fixed at ambient temperature for 1 h with 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, containing 5% sucrose. The cells were then incubated overnight at 4°C in 0.1 M sodium cacodylate buffer, pH

7.4, containing 7.5% sucrose, and then washed three times during a 15-min period in 50 mM Tris-HCl buffer, pH 7.4, with 7.5% sucrose. They were preincubated for 5 min in Tris-HCl buffer containing 1 mg/ml 3,3′-diaminobenzidine (Grade I, Sigma Chemical Co.) and then incubated for 10 min with diaminobenzidine containing 0.01% (vol/vol) H_2O_2. After being washed they were exposed to 1% unbuffered OsO_4 for 2 min and then postfixed in reduced OsO_4 (which was produced by adding 5 mg of $K_4Fe(CN)_6$ to 2 ml of 1% unbuffered OsO_4) for 20 min at 4°C. After the cells were rinsed for 2 min in 1% unbuffered OsO_4, they were dehydrated in alcohols, washed off the plates with propylene oxide, and prepared for transmission electron microscopy. Control procedures included substitution of nonimmune rabbit IgG or buffer for the primary antiserum.

Preparation of Platinum-Carbon Replicas

Platinum-carbon replicas of broken-open macrophages were prepared by either quick-freeze deep-etch or critical-point-dry methods, as described previously (1, 2). In brief, cells cultured on coverslips were broken open in inside buffer (100 mM KCl, 5 mM $MgCl_2$, 3 mM EGTA, and 20 mM Hepes, pH 6.8) by placing a poly-L-lysine-coated coverslip over them and pulling it away. For quick-freeze deep-etch, broken-open cells were fixed, rapidly frozen by contact with a copper block that had been cooled to liquid helium temperature (4°K) (25), freeze-dried in a Balzers freeze-fracture apparatus (Balzers, Hudson, NH), and then rotary replicated with platinum-carbon at an angle of 25°. For critical-point drying, broken-open cells were fixed with lysine, glutaraldehyde, OsO_4, and tannic acid, dehydrated through graded alcohols, critical-point dried out of bone-dry liquid CO_2, and rotary replicated (2). Replicas were examined in a JEOL 100CX or Philips 300 electron microscope at 80–100 kV.

Morphometric Analysis

For detection of clathrin basketwork assembly, photographs were taken randomly from large open areas that were not heavily obstructed by cytoskeleton and where plasma membrane was easily visible. Obstructed areas were excluded from the reference area. About 25 photographs of ~10 μm^2 each were taken from each sample, and the amount of clathrin-coated area and reference area was quantified with a digitizer (Hewlett-Packard Co., Palo Alto, CA). Preliminary studies indicated that after the areas in ~20 photographs were summed, the coefficient of variation of the percentage of coated areas became small. Because some of the coated patches were rounded, these coated areas were measured separately. Although it was not possible to measure the surface area of round coats directly, the area of round surfaces was estimated by multiplying the apparent area by two. The number of coated patches was determined from the same micrographs as those used for morphometric analysis. In addition, for some samples the number of coated patches only was determined from lower magnification micrographs of ~30 μm^2. Approximately 25 micrographs were used in each sample.

Results

Observations on Macrophages Freshly Explanted into Culture

Clathrin Is Redistributed During Adherence and Spreading of Freshly Harvested Macrophages on Immune Complex-coated Surfaces. For the study of clathrin redistribution during the spreading of macrophages on immune complex-coated surfaces, freshly harvested macrophages were attached to the coverslips at 4°C for 60 min, warmed to 37°C for various periods, and then fixed and stained with anticlathrin antibody and observed by indirect immunofluorescence. After 60 min of adhesion at 4°C, cells remained round with an area of contact of 200–300 μm^2 (Fig. 1), and punctate clathrin staining was observed throughout the cytoplasm (Fig. 2*a*). Shortly after warming to 37°C (4 min), the adherent area began to increase and cells began to assume a fried-egg appearance. Although a membrane veil had already started to spread out from the rounded macrophages, most of the punctate clathrin staining was still confined to the perinuclear area at this time (Fig. 2*b*). By 8 min clathrin staining had appeared in the peripheral veil (Fig. 2*c*). By 12–20 min the spread area had reached a maximum of 800–900 μm^2 (Fig. 1). When

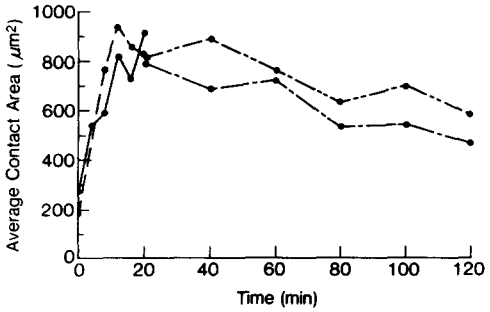

Figure 1. Time course of spreading of newly harvested macrophages on immune complexes. Coverslips were coated with 3 mg/ml BSA and then with 0.12 mg/ml mouse anti-BSA IgG. Macrophages were attached to the coverslips for 60 min at 4°C, then warmed at 37°C for 0–120 min. The spread area was determined by tracing the profiles of >100 cells for each sample on a digitizer. The data were collected from four different experiments, indicated by different lines. The standard error of each point was <5%.

viewed by interference-reflection microscopy, macrophages had extensive black areas at their spreading edges, which indicated close contact in these peripheral regions (data not shown). From 12 to 40 min, when the cells were fully spread, much of the punctate immunofluorescence was seen in the peripheral area, and perinuclear staining was depleted (Fig. 2, *d–f*). There was little change in the concentrated intracellular clathrin in cells plated on BSA alone, although some clathrin was seen in the small veil of spreading at 20 min. Cells just beginning to spread had many microvilli and folds on their surface, whereas fully spread cells were completely flattened and had few surface microvilli. After 40 min the spread area began to decrease, and by 2 h the cells became more heterogeneous in shape and reached a plateau of ~600 μm^2. By 80–120 min, most of the cells had regained clathrin staining in the perinuclear area, although peripheral punctate staining persisted (Fig. 2, *g–h*). By 24 h, when strong Golgi staining at one side of the nucleus had developed, abundant peripheral clathrin staining was still seen (Fig. 2*i*). Although the spread areas of cells were similar at 2 and 24 h, there was more clathrin in cells cultured for 24 h than in macrophages during their first 2 h in culture, which suggests that clathrin synthesis had taken place.

Ultrastructural Immunoperoxidase Observations Reveal the Redistribution of Clathrin from the Golgi Region During Ligand-induced Spreading. The distribution of clathrin in macrophages before and after spreading was investigated by antigen localization using a polyclonal antibody against clathrin and an immunoperoxidase procedure. In the nonspread cells, few coated vesicles were seen along the plasma membrane, and little immunoperoxidase staining was present (Fig. 3). This paucity of surface clathrin was observed both in cells fixed in vivo in the peritoneal cavity and in cells attached to immune complexes for 60 min at 4°C. Most of the staining for clathrin was seen on coated vesicles along and near the Golgi elements (Fig. 3). Abundant clathrin basketworks were also observed in the Golgi region by replica methods (data not shown).

When the macrophages were allowed to spread on immune complexes for 20–40 min at 37°C, there was a major redistribution of clathrin (Fig. 4). The clathrin staining in the Golgi region diminished (Fig. 4, *a* and *b*), and clathrin staining was

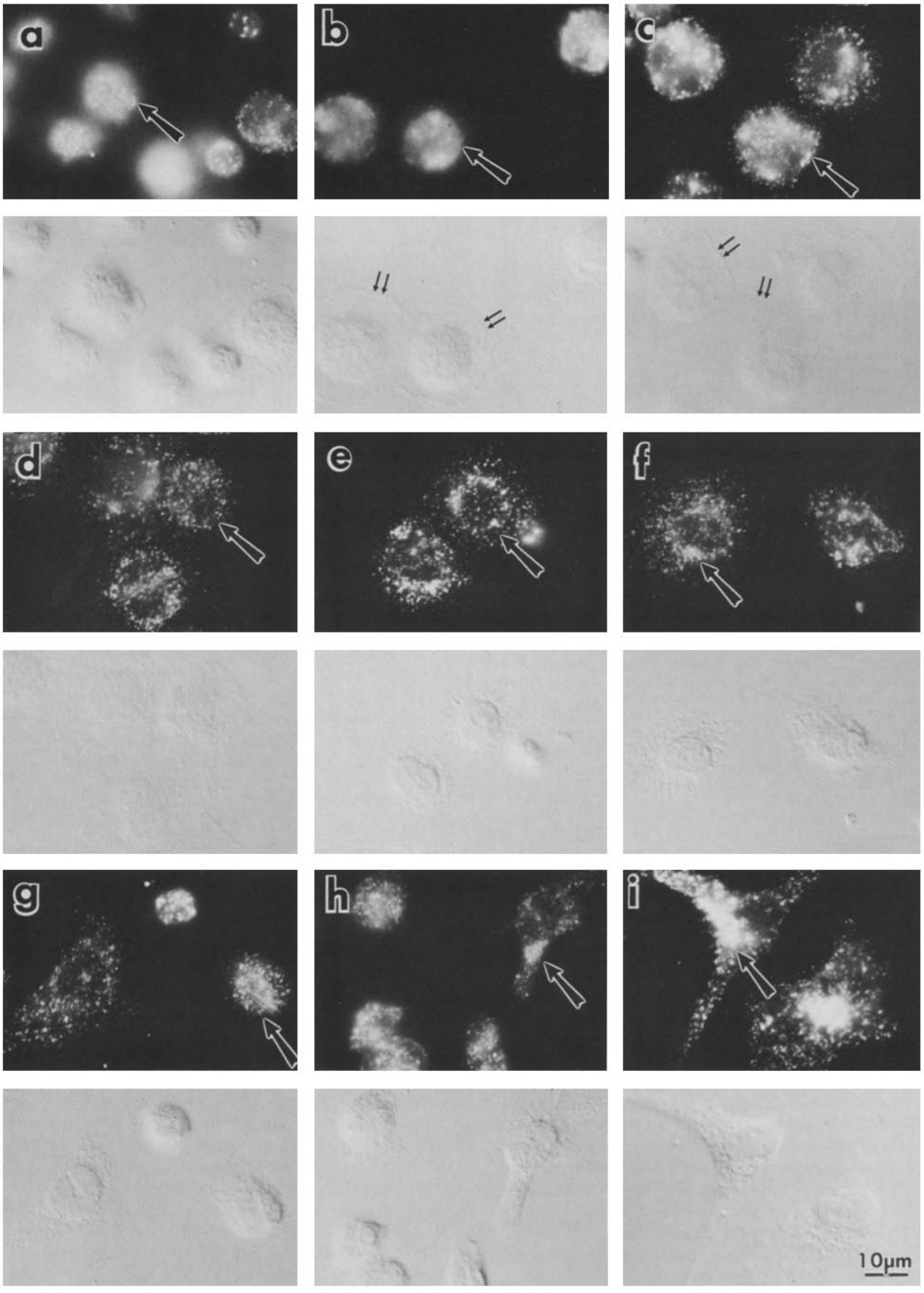

Figure 2. Redistribution of clathrin during initial spreading of freshly harvested macrophages on immune complexes. Macrophages were attached to the coverslips coated with BSA and 0.12 mg/ml mouse anti-BSA IgG for 60 min at 4°C, then warmed to 37°C for (*a*) 0, (*b*) 4, (*c*) 8, (*d*) 12, (*e*) 20, (*f*) 40, (*g*) 80, and (*h*) 120 min, and (*i*) 24 h, and stained with rabbit anticlathrin antibody. Matched pairs of immunofluorescent images and Nomarski-optic images are shown. The edges of the advancing plasma membrane are shown by small double arrows, and the clathrin staining in the perinuclear Golgi area by large arrows.

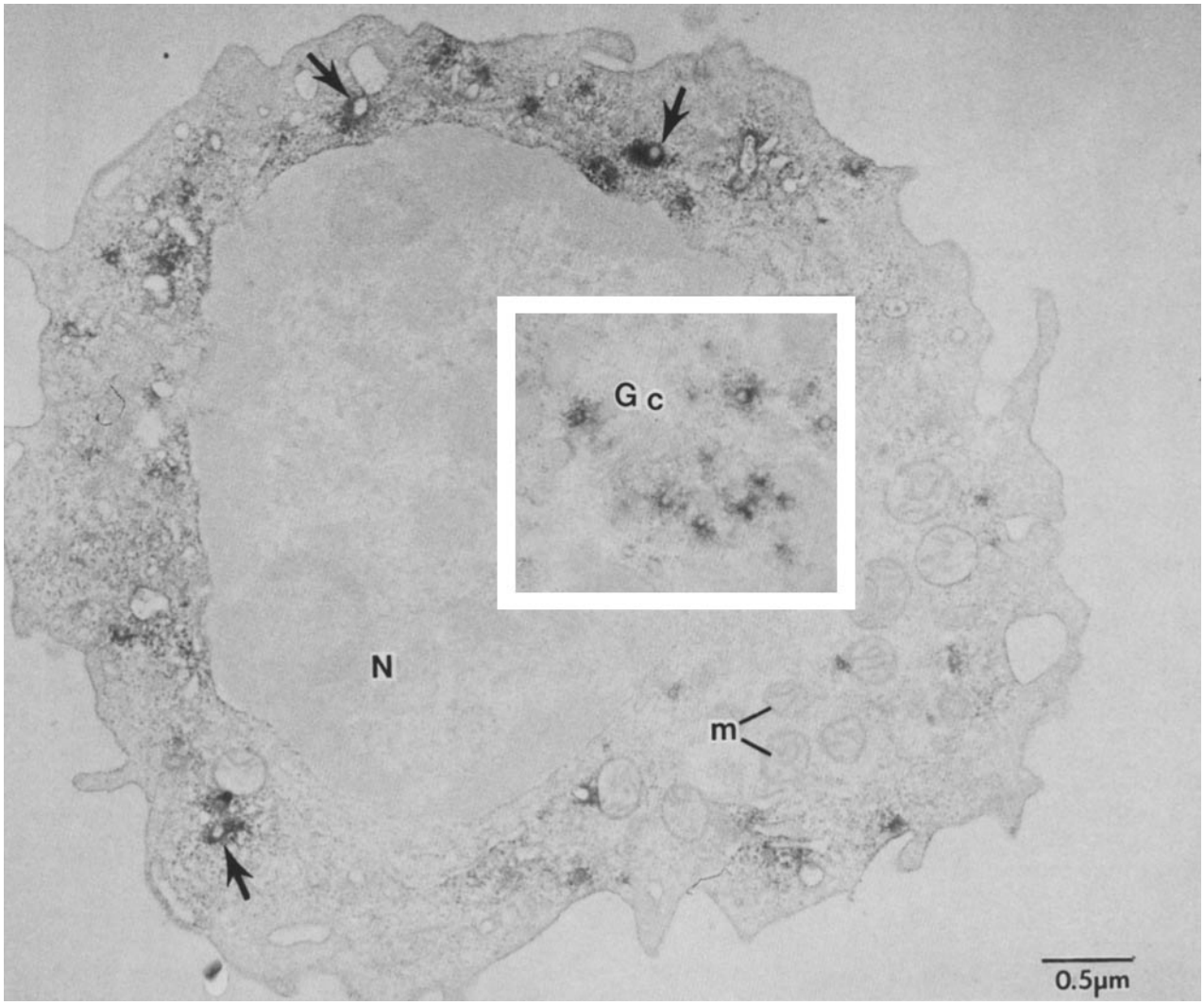

Figure 3. Electron micrographs of nonspread, resident peritoneal macrophages. Labeling of clathrin using an immunoperoxidase procedure on cells made permeable by saponin reveals dark reaction product on the cytoplasmic surface of vesicles, which are presumably coated (arrows). Most reactive vesicles are located in the Golgi region (*Gc*, inset), or cytoplasm, and few are on the plasma membrane. Note that the reaction product diffuses and spreads beyond the antigenic sites, a limitation of the immunoperoxidase procedure noted by Courtoy et al. (11). *N*, nucleus; *m*, mitochondria.

seen on both the nonadherent (Fig. 4*a*) and adherent (Fig. 4*c*) plasma membrane surfaces. The weaker staining of the adherent surface was probably due to the greater difficulty in penetration of anticlathrin antibodies to this region of the cell; however, abundant clathrin basketworks on the adherent surface were observed by replica procedures (Fig. 5).

Clathrin Assembly on Adherent Plasma Membrane Begins by 6 Min at 37°C in Freshly Harvested Macrophages. Clathrin basketworks on the inside adherent surface of macrophages spreading on immune complexes were studied in platinum-carbon replicas of broken-open cells. When suspended macrophages were attached to the immune complex–coated surfaces at 4°C for 60 min, no clathrin-coated patches were observed on the adherent membranes (Table I and Fig. 5*a*), although distinct cytoskeletal foci had developed (Figs. 5*a* and 6*a*). As macrophages were warmed to 37°C on immune complexes, clathrin-coated patches were not observed until 6 min (Fig. 6*c*), and abundant surface clathrin was seen later (Table I and Figs. 5*b* and 6, *d–f*). This contrasts with spreading, which was already under way by 4 min at 37°C (Figs. 1 and 2).

IgG Density That Induces Clathrin Assembly on Plasma Membrane Is Similar to the Density That Induces Spreading. To study whether the interaction of Fc receptors and immune complexes induces the clathrin-coated patches on plasma membrane, we performed quantitative analysis on cells spread on immune complexes. First, the relationship between the concentration of immune complexes and the extent of spreading was studied by coating BSA bound to coverslips with increasing amounts of anti-BSA IgG and examining the spreading of macrophages on them (Fig. 7). A few cells spread on BSA alone, but their contact area was always small (<500 μm^2). A significant increase in spreading was first observed when 3×10^{-3} mg/ml of IgG was used to form complexes. At this concentration the contact area of the spread cells was 800–900 μm^2 each, but only 20% of cells were spread (Fig. 7*a*). Interestingly, at $\geq 3 \times 10^{-2}$ mg/ml of IgG, the percentage of spread cells reached a maximum (>80%), but the spread

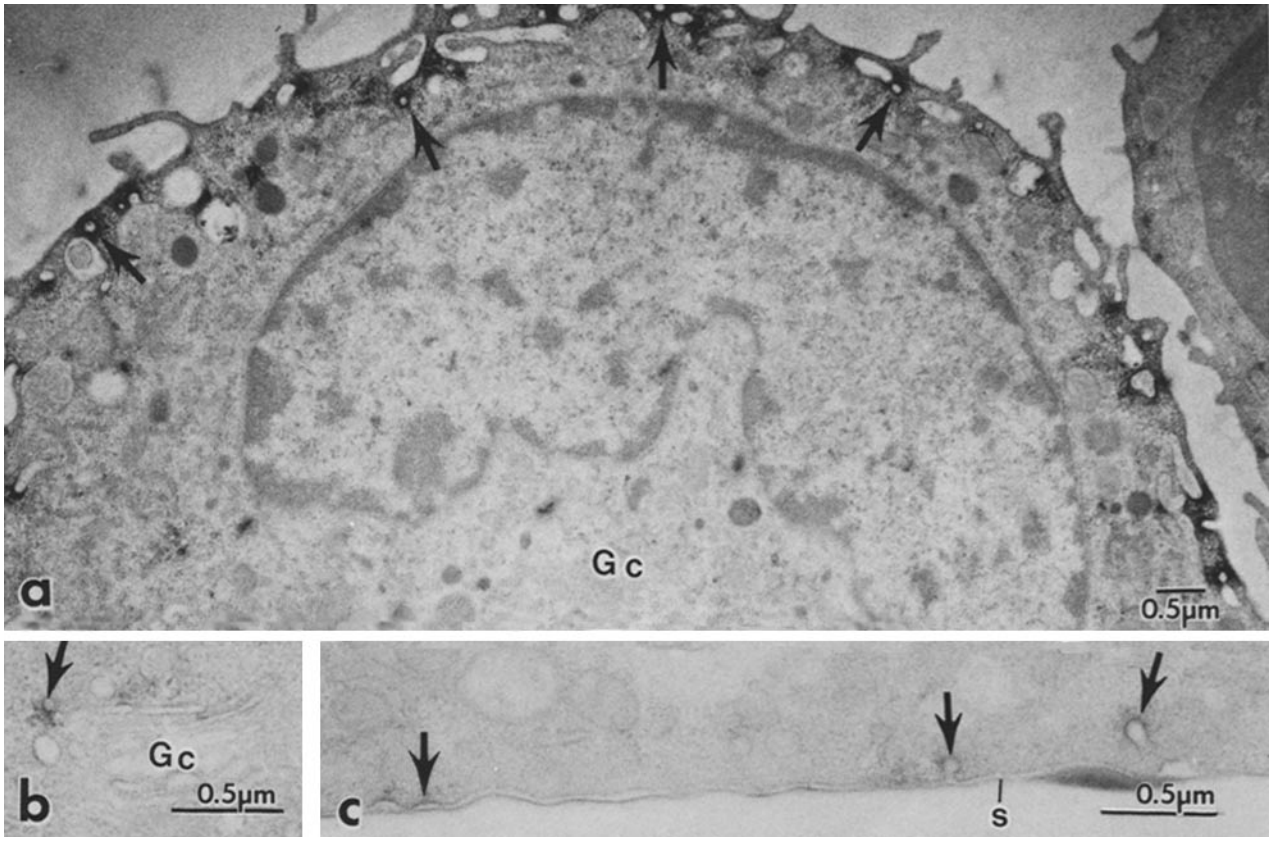

Figure 4. Electron micrographs of newly harvested macrophages spread on immune complexes for 20 min at 37°C. Cells were labeled with the anticlathrin antibody using an immunoperoxidase procedure. Note the dramatic redistribution of clathrin (arrows) from the Golgi region (*Gc*) of the cell to the adherent and nonadherent membranes. *s*, adherent surface.

area of the cells stayed the same (800–900 μm^2). When the amount of IgG present in immune complexes was quantified by preparing the coverslips using fluorescein-labeled anti-BSA IgG, it was evident that spreading of macrophages was induced at 300 IgG molecules/μm^2 and reached a maximum at 1,000 IgG molecules/μm^2 (Fig. 7*b*). The densities of IgG that induced spreading on BSA-anti-BSA immune complexes were similar to those that induced rosette formation and phagocytosis of IgG-coated erythrocytes (52; data not shown). Anti-BSA $F(ab')_2$ did not induce spreading, which indicated that spreading was dependent on the Fc portion of IgG. It was necessary to present IgG to macrophages as immune complexes, because anti-BSA IgG that was coupled directly to coverslips using poly-L-lysine and glutaraldehyde did not induce spreading, even at 10 times the necessary density of IgG.

When clathrin coats were quantified in spreading macrophages, a dose-dependent increase in the density of coated patches on the adherent membrane surface and in the total number of coated patches per cell was observed over the range of IgG concentrations that induced spreading (Table II). There was also a slight increase in the density of coated patches on the nonadherent top surfaces of the macrophages. At maximum spreading on immune complexes, there was a more than fourfold increase in the number of clathrin patches on the adherent surface as compared with cells spread on BSA alone (Table II), and a remarkable increase of >200-fold as compared with the macrophages freshly lavaged from the peritoneal cavity (Table I). These data support the concept that Fc receptor–mediated spreading induces clathrin assembly at the site of ligand-receptor interaction in macrophages, but other physiologic changes induced by placing cells in culture, presumably endocytic rate, may also have profound effects on clathrin distribution in macrophages.

Coated patches on the adherent plasma membrane varied in size from a few assembled hexagons of basketworks to large patches encompassing 8–10 coated vesicle equivalents. To take the patch size into account, the area of plasma membrane coated with clathrin basketworks was determined (Table III). Macrophages spread on BSA alone had 1.4% of the adherent surface coated with clathrin basketworks, whereas those spread on immune complexes had 3.5% of the adherent membrane coated (Table III). The density of clathrin basketworks on the nonadherent surface did not change when the macrophages were spread on the immune complexes, indicating that the increase in clathrin coats was primarily on the adherent plasma membrane surface (Tables II and III). However, because cells spread on BSA had only ~400 μm^2 of adherent plasma membrane, whereas cells spread on immune complex–coated surfaces had ~800 μm^2, the actual increase in clathrin basketworks was fivefold (5.6 to 28 μm^2). These data suggest that the site of ligand-receptor interaction is important for clathrin assembly. Clathrin coats were not distributed uniformly over the adherent surface of the macrophages, although large areas were coated (Fig. 5).

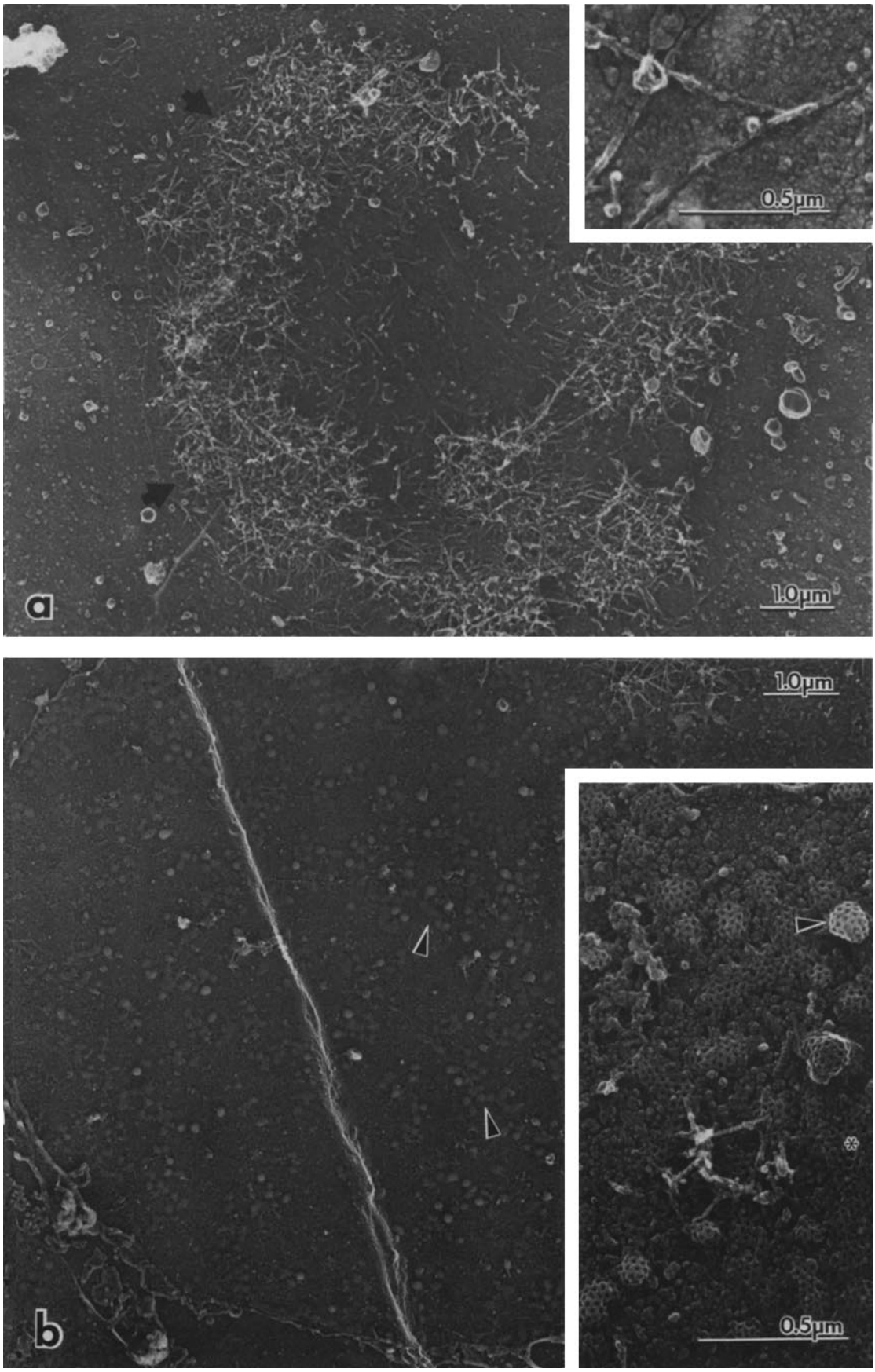

Figure 5. Adherent surface of newly harvested macrophages viewed from the cytoplasmic side in platinum-carbon replicas of critical point–dried broken-open cells. (*a*) Macrophages were attached to the immune complex–coated surface formed from 3 mg/ml BSA followed by 3 mg/ml of rabbit anti-BSA IgG for 60 min at 4°C. Foci-like cytoskeletal structures were present near the rim of the exposed membrane (arrows), but no clathrin was observed. (*b*) Macrophages were spread on the immune complex–coated surface for 20 min at 37°C. Numerous coated patches (arrowheads) can be seen. Many of the coated patches are made up of areas comprising several fused coated vesicle equivalents (asterisk).

Observations on Cultured Macrophages

Up to this point our study on redistribution of assembled clathrin by ligand-receptor interaction was made with macrophages freshly explanted into culture, which initially are unspread and have little surface clathrin. We were interested in comparing the clathrin kinetics in those cells with that in spread, cultured macrophages, which already have abundant surface clathrin (1) and quantitatively increased total clathrin, as determined by immunocytochemistry (Fig. 2*i*). In addition, because most previous work on endocytosis and clathrin dynamics in macrophages (1–3, 30, 33, 36) or fibroblasts (5, 19, 28) has used cells in culture, an analysis of clathrin distribution in response to frustrated phagocytosis of immune complexes would be most directly comparable to these studies.

Table I. Time Dependence of Clathrin-coated Patch Assembly Observed in Freshly Explanted Macrophages Spreading on Immune Complexes

Time at 37°C	Clathrin-coated patches per μm	Estimated adherent surface area per cell	Total coated patches on adherent surface per cell
min	*n*	μm^2	*n*
0	<0.01* (12)	200	<10
20	2.4 ± 0.2 (23)	800	1,920

Macrophages were attached to coverslips coated with 3 mg/ml BSA and with 0.3 mg/ml of rabbit anti-BSA IgG at 4°C for 60 min, and then warmed to 37°C for 0 or 20 min. Bottoms of broken-open macrophages were fixed, critical point–dried, and replicated. Coated patches were counted in representative micrographs of 10–30 μm^2 of unobstructed membrane. Data are expressed as the mean number of coated patches per square micrometer of membrane ± standard error, with number of micrographs in parentheses. The adherent surface area, estimated from Fig. 1, was multiplied by the number of clathrin-coated patches per square micrometer to obtain the total number of coated patches and pits on the adherent surface. The data are taken from three independent experiments. Some of the coated patches were large, consisting of up to 10 individually discernible coated pits (Fig. 5*b*, inset). If these are taken into account, the total number of coated patches increases by 15–30%.

* Only one coated patch was observed in 170 μm^2 of adherent surface area.

Clathrin Is Reversibly Redistributed in Cultured Macrophages Spreading on Immune Complex–coated Surfaces. We first studied the distribution of clathrin in macrophages that already had substantial amounts of surface clathrin basketworks by being placed in culture for 24 h. Such macrophages that have attached to and spread on coverslips coated with BSA during 24 h in culture can be induced to spread further: immune complexes are formed under the cells at 4°C by their being incubated with anti-BSA IgG by the underlay method and then warmed to 37°C. Macrophages cultured on BSA alone for 24 h appeared flattened, with a spread area of 600–900 μm^2 (Fig. 8), and had strong clathrin staining concentrated in the Golgi area (Fig. 9*a*). When these cells were incubated with anti-BSA IgG at 4°C, the edges of the membranes ruffled, which indicates that even at 4°C the membranes were dynamic; although distribution of clathrin in the Golgi region changed little, there appeared to be some additional peripheral staining in these cells (Fig. 9*b*). As soon as the cells were warmed to 37°C, they became flattened, increasing their area of contact with the coverslip to 1,100–1,200 μm^2 by 4–8 min (Fig. 9, *c* and *d*). Clathrin staining in the Golgi area was rapidly depleted (as early as 4 min) (Fig. 9, *c–g*). After 80–120 min the strong staining of clathrin began to reappear in the Golgi area in some cells (Fig. 9, *h* and *i*). At this time cells altered their morphology and reassumed shapes similar to those of cells cultured on BSA for 24 h. That redistribution was not blocked by cycloheximide suggests that synthesis of new clathrin was not required, at least in the first 1–2 h.

Clathrin Assembly Occurs at 4°C in Cultured Macrophages. When coated patches were observed on the adherent membrane of macrophages cultured on BSA for 24 h, their density was found to be similar to that of patches on newly adherent macrophages spread on BSA for 20 min (Fig. 10*a*

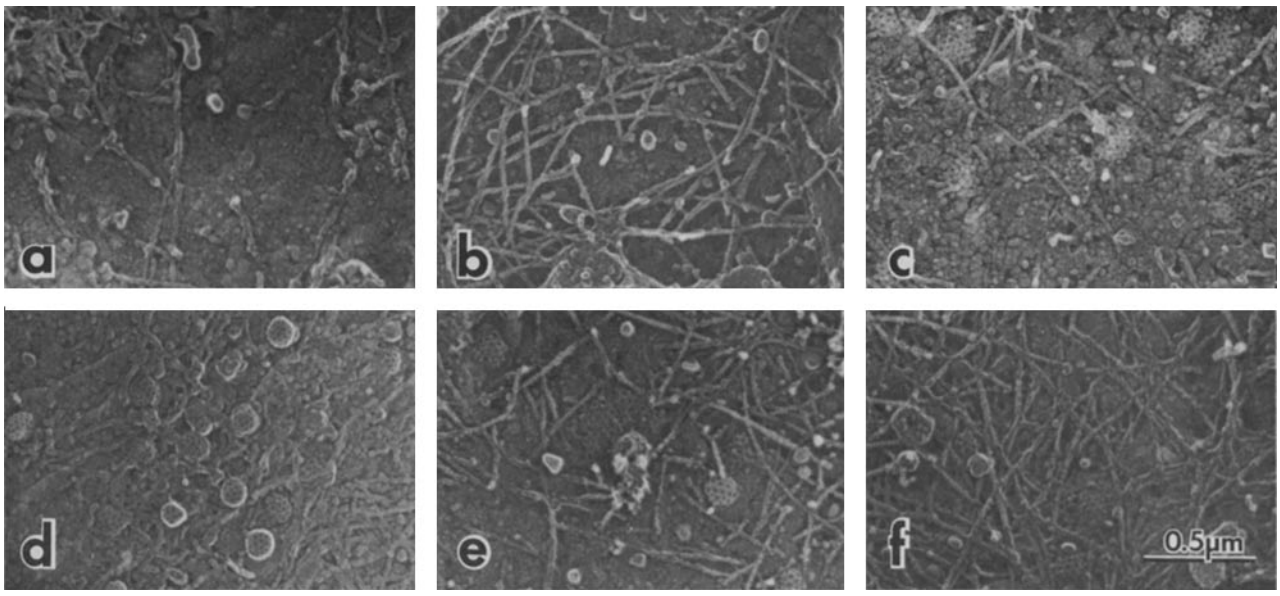

Figure 6. Clathrin-coated patches on the adherent plasma membrane of newly harvested macrophages spreading on immobilized immune complexes observed by platinum-carbon replicas of critical point–dried cells. Macrophages were attached to the immune complex–coated surface for 60 min at 4°C and then spread at 37°C for (*a*) 0, (*b*) 2, (*c*) 6, (*d*) 20, (*e*) 60, or (*f*) 120 min. Coated patches were observed at 6–120 min but not at 0 and 2 min.

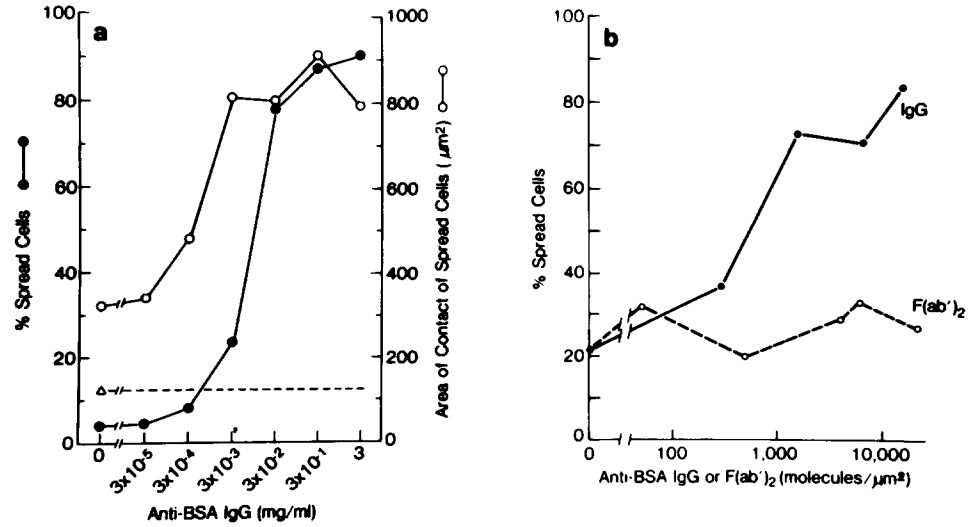

Figure 7. Dose response for spreading of macrophages on immune complexes. (*a*) Dependence of macrophage spreading on the concentration of anti-BSA IgG used to form immune complexes. Coverslips were coated with 3 mg/ml BSA and then with 0–3 mg/ml rabbit anti-BSA IgG. Freshly harvested macrophages were spread for 40 min at 37°C. The adherent area of spread cells (○) was determined by a digitizer. Profiles of 20 cells were traced for each sample. The bar indicates the standard error of the mean. The area of contact of unspread cells (△) is also indicated. The percentage of spread cells (●) was determined by differential counting of spread and unspread cells (>100 total). The data point for 3 mg/ml anti-BSA IgG is from a separate experiment. (*b*) Dependence of macrophage spreading on IgG or $F(ab')_2$ density. The number of IgG or $F(ab')_2$ molecules bound to coverslips was determined as described in Materials and Methods. The percentage of spread cells was determined as described in *a*.

Table II. Dose Response of Clathrin-coated Patches Observed in Freshly Explanted Macrophages Spreading on Immune Complexes

	Clathrin-coated patches per μm		Total coated patches per cell	
Anti-BSA IgG	Nonadherent surface*	Adherent surface	Nonadherent surface*	Adherent surface
mg/ml	*n*	*n*	*n*	*n*
0	0.3 ± 0.1 (25)	0.9 ± 0.2 (24)	120	360
0.003	0.6 ± 0.1 (25)	1.3 ± 0.3 (27)	300	650
0.3	ND‡	2.4 ± 0.2 (23)	ND	1,920
3.0	0.6 ± 0.1 (22)	2.1 ± 0.6 (26)	480	1,680

Macrophages were attached to coverslips coated with 3 mg/ml BSA and various concentrations of rabbit anti-BSA IgG at 4°C for 60 min, then warmed to 37°C for 20 min. Both tops and bottoms of broken-open macrophages were fixed, critical point-dried, and replicated. For details of quantification, see Table I. The numbers of clathrin-coated patches per square micrometer on the adherent surface of cells spread on BSA alone were significantly different ($P < 0.05$) from the values for cells spread on immune complexes.

* When estimating the nonadherent surface area by light microscopy, we did not take curvature, surface ruffles, and microvilli into account; therefore, the true area is underestimated by a factor of from ~1.5 (for spread cells) to 3 (for unspread cells).

‡ ND, not determined.

and Table III). After immune complexes were formed under the cultured macrophages at 4°C for 60 min, an increase in microfilament organization and a two- to threefold increase in the number of clathrin-coated patches (Fig. 10*b* and Table IV) was observed on the adherent membrane of most of the cells (>80%). This contrasts with the observation that although there was also an increase in cytoskeletal organization after initial attachment of freshly lavaged macrophages at 4°C (Fig. 5*a*), few clathrin basketworks were present after binding to immune complexes at 4°C (Fig. 5*a* and Table I). It therefore appears that cultured macrophages can assemble clathrin more readily than macrophages freshly harvested from the peritoneum. After the cells were warmed for 20 min at 37°C, many coated patches were observed (Fig. 10*c*), and their density was similar to that of patches on cells spread on immune complexes for 20 min by the initial spreading method (Tables III and IV) and somewhat less than that of patches on cells that had not been warmed (Table IV).

Discussion

In this report we have described dynamic changes in the distribution of morphologically recognizable assembled clathrin during Fc receptor–mediated frustrated phagocytosis. The kinetics of clathrin redistribution are summarized diagrammatically in Fig. 11. We found that clathrin-coated patches assemble on the plasma membrane adherent to immune complexes. In cultured macrophages, clathrin assembled rapidly on the membrane, even at 4°C, whereas in macrophages freshly lavaged from the peritoneal cavity, there was a lag of 6 min at 37°C before clathrin appeared, suggesting an additional rate-limiting step. That the assembly of clathrin was dependent on ligand concentration indicates that the receptor-ligand interaction was involved. Although the immune complexes induced both spreading and clathrin assembly at the plasma membrane, the two processes were separated kinetically. Spreading preceded clathrin assembly at the cell surface in newly harvested macrophages but followed clathrin assembly in cultured macrophages, which suggests that they are not causally related. Concomitantly, clathrin from the Golgi region of the macrophages diminished, and then was restored by 2 h.

One unexpected observation was the low number of clathrin-coated patches on the plasma membrane of resident macrophages both in vivo and freshly harvested from the peritoneal cavity and attached to immune complexes at 4°C. Al-

Table III. Comparison of Membrane Area Coated with Clathrin Observed in Freshly Explanted and Cultured Macrophages Spreading on Immune Complexes

Macrophage	Anti-BSA IgG	Clathrin-coated surface area (% of total)		Estimated surface area per cell	Total clathrin-coated surface area per cell	
		Nonadherent surface	Adherent surface		Nonadherent surface	Adherent surface
	mg/ml			μm^2	μm^2	μm^2
Freshly explanted	0	0.8 ± 0.2 (25)	1.4 ± 0.4 (24)	400	3.2	5.6
	3.0	0.6 ± 0.2 (23)	3.5 ± 0.8 (26)‡	800	4.8	28
Cultured for 24 h	0	ND*	1.5 ± 0.4 (23)	900	ND	14
	0.3	ND	3.1 ± 0.5 (26)‡	1,200	ND	36

For freshly explanted macrophages, coverslips were coated with 3 mg/ml BSA and then with 3 mg/ml of rabbit anti-BSA IgG. Cells were attached for 10 min at room temperature and then spread for 20 min at 37°C. Both tops and bottoms of broken-open macrophages were fixed, critical point–dried, and replicated. For cultured macrophages, cells were cultured for 24 h on coverslips coated with 3 mg/ml BSA, and then the coverslips were incubated with 0.3 mg/ml of rabbit anti-BSA IgG for 60 min at 4°C. After unbound IgG was washed off, macrophages were incubated for 20 min at 37°C. The bottom membranes of macrophages were fixed, critical point–dried, and replicated. For quantification, micrographs with 5–10 μm^2 of membrane that was unobstructed by cytoskeleton at a final magnification of 70,000 (freshly harvested cells), or 10–20 μm^2 at 50,000 (cultured cells) were analyzed. The percentage of membrane area coated with clathrin, corrected for coated pits, is expressed as the mean ± standard error with the number of micrographs in parentheses. The surface area of either nonadherent or adherent membrane of cells was estimated from Figs. 7 and 8 (however, see footnote to Table II). The percentage of membrane coated with clathrin was multiplied by the estimated surface area to obtain total clathrin-coated surface area per cell.

* ND, not determined.

‡ Significantly different from values obtained with spreading on BSA alone ($P < 0.05$; two-sample t test).

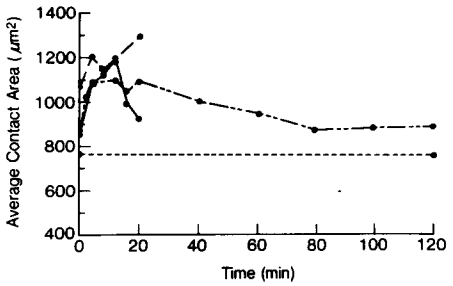

Figure 8. Time course of spreading of macrophages cultured on immune complexes. Macrophages were cultured on BSA-coated coverslips for 24 h, then incubated with 0.12 mg/ml mouse anti-BSA IgG for 60 min at 4°C. After unbound IgG was washed off, macrophages were warmed to 37°C for 0–120 min. The spread area was determined as described in Fig. 1. The data were collected from four different experiments indicated by different lines. The horizontal dashed line indicates the mean spread area of cells cultured on BSA alone obtained from three experiments.

though there were remarkably few cell surface–associated clathrin pits on these cells, assembled clathrin (observed by immunocytochemistry and platinum replica procedures) was concentrated intracellularly in the Golgi region.

These findings suggest that if surface clathrin coats reflect endocytosis, then resident peritoneal macrophages in vivo are relatively inactive in receptor-mediated endocytosis in the absence of challenge by specific ligands. In support of this hypothesis, it has been observed that previtellogenic mosquito oocytes have large intracellular stores of clathrin but little surface clathrin; however, once endocytosis of yolk proteins commences, abundant surface clathrin appears (46, 47). Incubation of the macrophages in PBS used for lavage did not induce assembly of clathrin coats. The cells began to spread immediately at 37°C, but there was a lag period of 6 min before clathrin patches appeared at the cell surface. Once placed in culture in an amino acid–rich, protein-free culture medium, pinocytic activity by the macrophages may be rapidly initiated by substances in culture medium and/or materials secreted by the cells. By 20 min the clathrin concentrated in the Golgi region had diminished. The increase in coated patches after 20 min at 37°C was at least 30-fold, even on the nonadherent surface of the attaching macrophages, and was as much as 200-fold on plasma membrane surfaces adherent to immune complexes.

Clathrin appears to be in a dynamic state and can be induced to assemble in increased amounts at the cell surface in many types of cells (1, 10, 15, 36, 38, 47, 48). Clathrin is usually seen within cells in a membrane-associated assembled basketwork structure (1, 4, 17, 59); however, unassembled clathrin triskelions stored in the cytoplasm are probably the source for the induced assembly at the plasma membrane (20, 46). Large aggregates of unassembled clathrin (46) have not been observed in most cells; nonetheless, diffusely distributed unassembled forms have been detected by immunocytochemistry (26), and recent estimates indicate that ~30–50% of cellular clathrin is in its unassembled form in a wide variety of cell types (20). Thus, clathrin is probably redistributed to macrophage plasma membranes from intracellular pools.

Our data on clathrin distribution in macrophages in vivo have important implications for the extrapolation of in vivo rates of membrane turnover, receptor turnover, fluid uptake, and membrane trafficking from rates derived from cells in culture (29, 30, 50). Previous studies have demonstrated relatively few plasma membrane–coated vesicles in macrophages in vivo (3) except in specialized sites such as regions in which macrophages interact with Leydig cells (35). That mutant cells defective in endocytosis require only ferric ions to proliferate (27) suggests that transferrin may be the only normal metabolite that must be taken into cells by endocytosis. Resident macrophages do not proliferate (56), have very few transferrin receptors (22), and thus may be endocytically inactive in the absence of specific ligands. However, clathrin coats are abundant in cells actively engaged in endocytosis in vivo (37) and in culture (1, 3, 36). In previous studies, concentrations of clathrin-coated pits and vesicles have been observed on the basal surfaces (28) or trailing edges (43) of cells, which suggests that endocytic activity may occur preferentially in these areas.

The redistribution of clathrin during Fc receptor–mediated spreading was not confined to the freshly harvested macro-

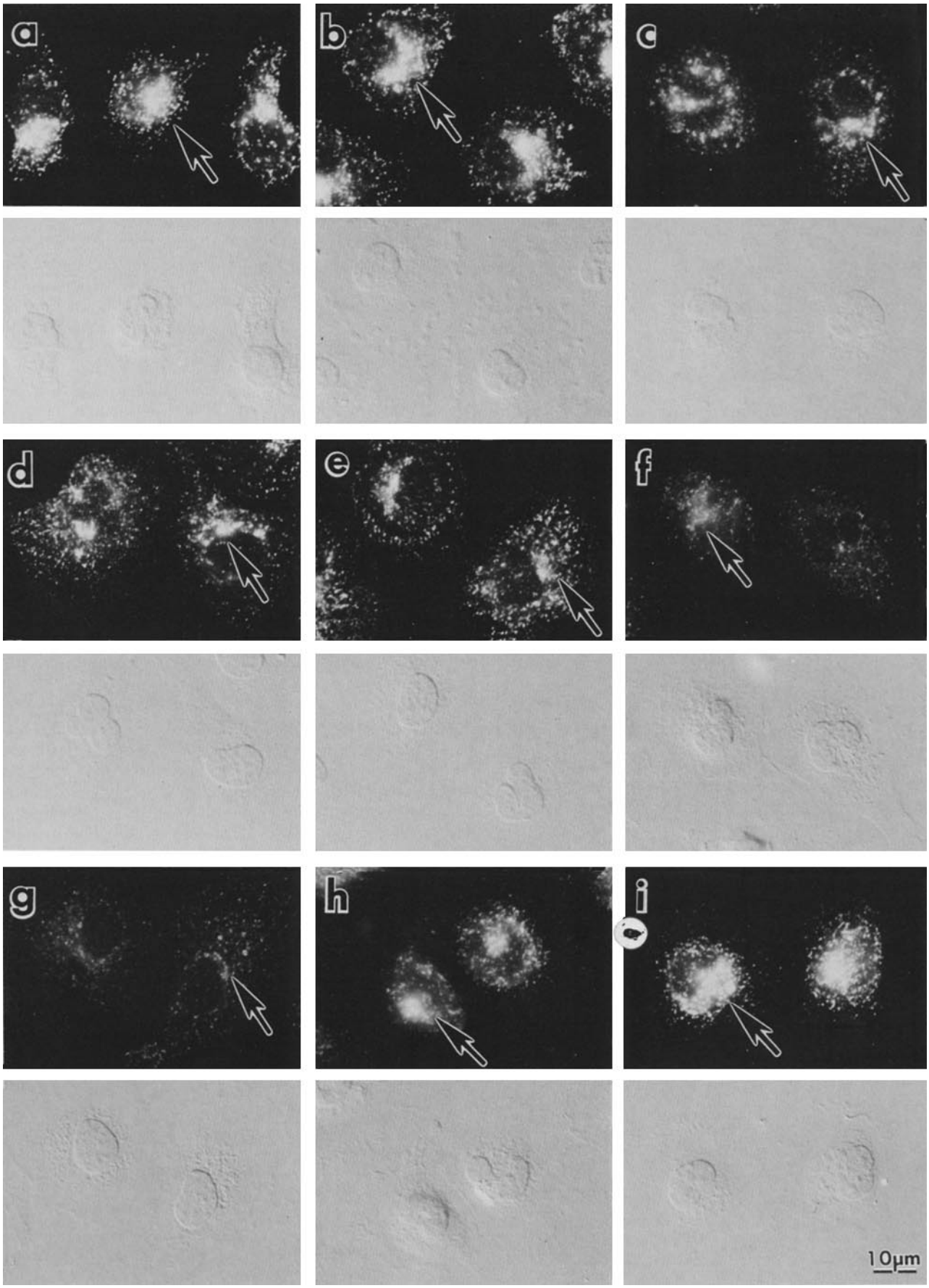

Figure 9. Redistribution of clathrin during spreading of cultured macrophages on immune complexes. Macrophages were cultured on BSA-coated coverslips for 24 h (*a*); then 0.12 mg/ml mouse anti-BSA IgG was added at 4°C for 60 min. Cells were then warmed to 37°C for (*b*) 0, (*c*) 4, (*d*) 8, (*e*) 12, (*f*) 20, (*g*) 40, (*h*) 80, and (*i*) 120 min, and stained with rabbit anticlathrin antibody. Matched pairs of immunofluorescent images and Nomarski-optic images are shown. The clathrin staining in the Golgi area is shown by arrows.

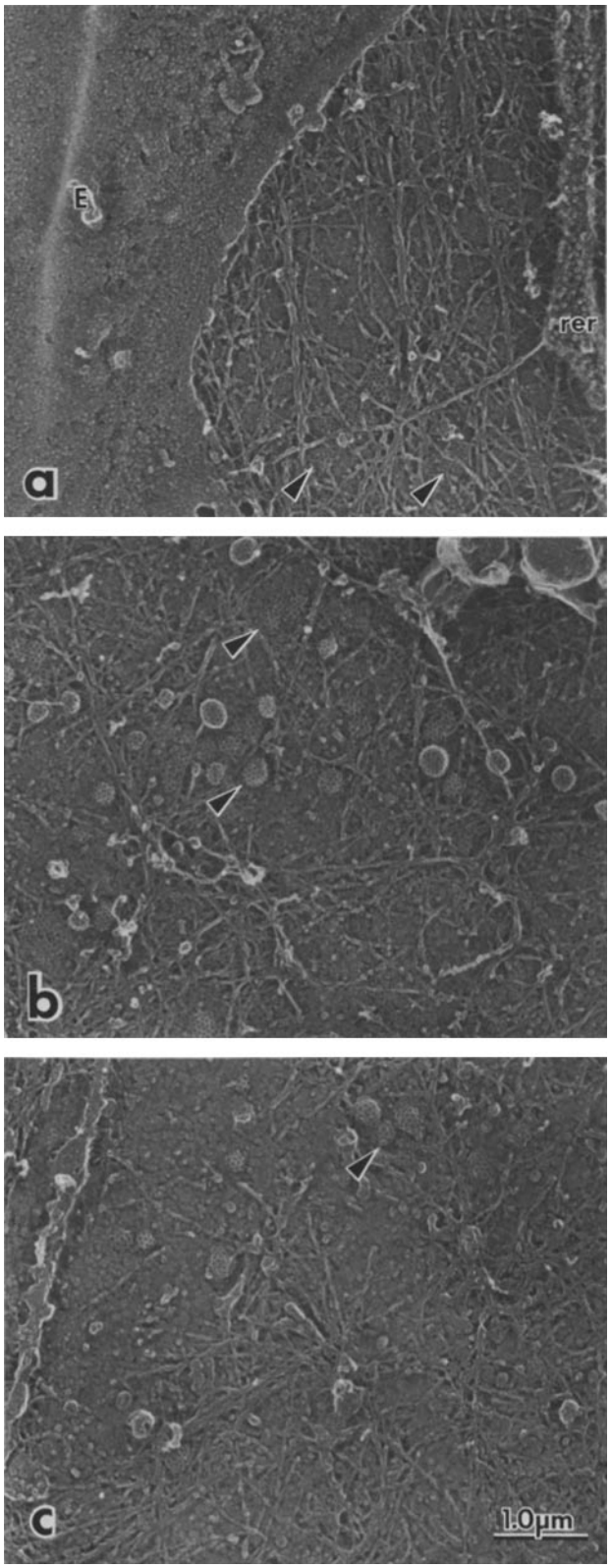

Figure 10. Assembly of coated patches on adherent membranes of cultured macrophages spread on immune complexes observed by platinum-carbon replica techniques. Macrophages were cultured for 24 h on BSA-coated coverslips, then incubated with 0.3 mg/ml rabbit anti-BSA IgG for 60 min at 4°C and for 20 min at 37°C. Some coated patches (arrowheads) are observed on the adherent surface of macrophages cultured on BSA alone for 24 h (*a*), whereas coated patches were greatly increased on the adherent surface of macrophages cultured with anti-BSA IgG for 60 min at 4°C (*b*). Abundant coated patches can also be seen on the adherent surface of macrophages subsequently incubated for 20 min at 37°C (*c*).

phages, and, therefore, was not solely involved in a mechanism for initiating clathrin assembly at the plasma membrane. When macrophages were cultured for 24 h on BSA-coated coverslips and then incubated with anti-BSA IgG, the assembly of clathrin coats at 4°C was observed before spreading (Fig. 11). One previous study indicated that an arrest of endocytosis by cold treatment did not alter the number of coated vesicles seen at the cell surface (19); however, in another study twice as many coated vesicles were seen immediately after warming of cells that bound low-density lipoproteins in the cold than under steady state conditions (4). Our data clearly demonstrate that the signal for clathrin assembly can be transduced in the cold via the Fc receptor. Clathrin also assembles within 2 min on latex phagosomes in cultured macrophages (1). The striking contrast between the lack of assembly of clathrin coats at 4°C followed by a 6-min delay in clathrin assembly at 37°C in newly harvested macrophages, on one hand, and rapid assembly of clathrin coats at 4°C in cultured macrophages, on the other hand, suggests that a fundamental change in the plasma membrane takes place during culture of macrophages. The possibilities for this change include (*a*) an alteration of membrane fluidity, allowing migration of proteins in the plane of the membrane, such as that observed for the complement receptor (21); (*b*) an alteration in the state of the Fc receptor so that the signal for clathrin coat assembly is more rapid; or (*c*) a rate-limiting requirement for assembling or modifying clathrin-associated proteins (40, 42) at sites that bridge the plasma membrane and the clathrin basketwork.

Clathrin Redistributes During Frustrated Phagocytosis. What is the reason for the clathrin redistribution during frustrated phagocytosis? Several observations by others on the nature of the clathrin-coated compartments related to the Golgi complex are instructive. Brown and his colleagues (7, 8) have shown that mannose-6-phosphate receptors accumulate in coated vesicles along Golgi cisternae in ligand-deficient cells, at the presumptive sorting site. Similarly, a clathrin-coated Golgi-related compartment accumulates in proinsulin-secreting cells when secretion is blocked by monensin, presumably at a sorting site (39). In both cases, the steady state distribution of clathrin has been perturbed by disruption of the normal intracellular traffic through the Golgi complex. In frustrated phagocytosis the rapid addition of membrane to the cell surface may also disturb intracellular traffic, thus increasing the turnover of membrane-associated clathrin from the Golgi region (Takemura, R., P. E. Stenberg, D. F. Bainton, and Z. Werb, manuscript submitted for publication), with a resulting decrease in assembled clathrin in that region. A redistribution of other membrane compartments such as secretory granules and endosomes communicating with the plasma membrane takes place concomitantly with clathrin redistribution during the time before establishment of a new steady state, when intracellular traffic of membrane is balanced. Secretion products are also directed to the adherent surface engaged in frustrated phagocytosis (24; Werb, Z., and D. F. Bainton, manuscript in preparation).

Table IV. Quantification of Clathrin-coated Patches Observed on Adherent Surfaces of Cultured Macrophages Spreading on Immune Complexes

Anti-BSA IgG	Time at 37°C	Total area of samples	Clathrin-coated patches counted	Clathrin-coated patches per μm^2	Estimated adherent surface area per cell	Total coated patches on adherent surface per cell
mg/ml	*min*	μm^2	*n*	*n*	μm^2	*n*
0		526 (24)	540	1.0	800	820
0.3	0	868 (40)	1,925	2.2	1,000	2,220
0.3	20	625 (27)	1,073	1.7	1,200	2,060

Macrophages were cultured on coverslips coated with 3 mg/ml BSA for 24 h, and then incubated with 0.3 mg/ml rabbit anti-BSA IgG at 4°C for 60 min. After the unbound IgG was washed off, the macrophages were warmed to 37°C for 0 or 20 min, and the bottom surfaces of the cells were fixed, critical point–dried, and replicated. Coated patches were counted in representative micrographs of 20–30 μm^2 of unobstructed membrane. The number of micrographs is shown in parentheses. The adherent membrane surface area, estimated from Fig. 8, was used to calculate the total number of coated patches on the adherent surface. Data are derived from two independent experiments. Some of the coated patches were large, consisting of up to 10 fused, individually discernible coated pits. If these are taken into account, the total number of coated patches increases by 10–35%.

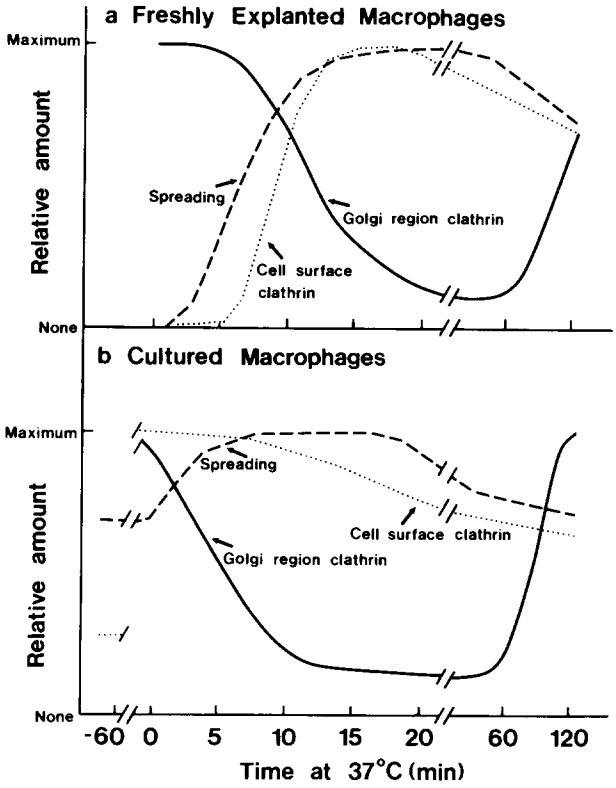

Figure 11. A diagrammatic summary of spreading and clathrin distribution in macrophages adhering and spreading on immobile immune complexes at 37°C. The data are qualitative and are deduced from the results. (*a*) In freshly explanted macrophages, spreading precedes clathrin assembly at the cell surface, and Golgi region clathrin redistribution is reversible. (*b*) In cultured macrophages the point at −60 min represents cells cultured on BSA for 24 h; then anti-BSA IgG was added at 4°C. The interval between −60 and 0 min represents the 60 min at 4°C. Note that clathrin associates with the cell surface at 4°C and precedes spreading, and that spreading and clathrin redistribution occur more rapidly than in freshly harvested cells shown in *a*.

Because a large amount of cellular clathrin is found as coated vesicles located away from the plasma membrane, especially deep in the cytoplasm and around the Golgi complex, clathrin may also be supplied to the cell surface by the redistribution of coated vesicles deep in the cytoplasm. Despite the fact that coated vesicles are believed to be involved in the intracellular transport of membranes and proteins (41), there have been only a few cases in which such movement of coated vesicles has been demonstrated directly (17, 18). Because of the delay in redistribution of clathrin from the Golgi region, as compared with the assembly of clathrin on the adherent surface and the resultant increase in surface area, there is probably not a direct movement of assembled clathrin-coated vesicles. It is more likely that a soluble pool of clathrin triskelions is the intermediate in redistribution, and this mechanism probably accounts for the marked assembly of clathrin on the adherent surface of cultured macrophages spreading on immune complexes at 4°C. It is apparent that tissues that are more endocytically active have smaller pools of unassembled clathrin (20). Studies are in progress to determine the distribution of clathrin between assembled and soluble pools in macrophages during frustrated phagocytosis.

Fc Receptor Interactions Signal Clathrin Basket Assembly in Macrophages. We observed a two- to threefold increase in density of clathrin coats in cells spread on immune complexes as compared with cells spread on BSA alone. When the increase in the total plasma membrane in spread macrophages is taken into account, the increase in coated structures was fivefold. It has been estimated that 0.2–0.8% of cellular protein of macrophages is clathrin (3, 20). Assuming that macrophage protein is ~100 $\mu g/10^6$ cells, each cell would have enough clathrin to cover between 80 and 200 μm^2 of membrane surface (12). The maximum area covered by clathrin basketworks on the plasma membrane of spreading macrophages used in this study was ~30 μm^2 per cell; therefore, nearly half the clathrin in these cells was assembled at the plasma membrane. Macrophages have ~1.3×10^5 Fc receptors (57), most of which are captured at the bottom surface during spreading onto immune complexes (14, 32–34). At 3 mg/ml of anti-BSA IgG, the density of the immune complexes on the dishes was ~10,000 molecules/μm^2; therefore, the average spacing of IgG molecules was ~10 nm, which is comparable to the distance between the vertices of the clathrin basketwork. Furthermore, although the average percentage of coated area of the cells spread on immune complexes was only 3.5%, some regions of the cells were coated up to 17% (for examples see Fig. 5*b*). Such dense concentrations of clathrin coats were never observed in control cells. Because the half-life of coated vesicles involved in receptor-mediated endocytosis is only 2–3 min at 37°C (5, 58), one interpretation is that regions with the highest density of clathrin coats are temporal foci for basket assembly. Although membrane spreading preceded the appearance of assembled clathrin at the plasma membrane by several minutes, we cannot rule out the possibility that the act of cell spreading on a substratum

may itself trigger clathrin assembly, even without defined ligands or receptors. Extensive assembled clathrin has also been observed at attachment sites of fibroblasts (28).

The frustrated phagocytosis system modeled Fc receptor-mediated phagocytosis well. IgG concentrations inducing phagocytosis were the same as those inducing spreading. The time course of macrophage spreading corresponded to that for the disappearance of Fc receptors from the upper surface observed previously in cultured macrophages (32). The fact that discrete coated vesicles are seen at the sites of close interaction of IgG-coated erythrocytes with macrophages (3, 36), rather than the large planar areas at sites of attachment of latex particles (1), suggests that Fc receptors may be clustered. Macrophage Fc receptors are internalized and degraded after attachment to mobile immune complexes (29, 30). The precise fate of Fc receptors in macrophages spreading on immobilized immune complexes remains to be determined. Spreading of cultured macrophages is also induced by agents, such as phorbol diesters (44), that do not act selectively at the adherent surface. It will be interesting to determine if such stimuli also induce clathrin assembly.

Why does clathrin assemble at the sites of membrane-receptor interaction? Because membrane spreading and clathrin assembly at the adherent surface of the macrophage are separated in time (Fig. 11), the signals transduced by ligand-receptor interactions that induce membrane spreading must be separate from those that induce clathrin assembly. Clathrin assembly is probably part of a response for receptor-mediated endocytosis and retrieval of membrane from the sites of interaction. These areas may represent clustering of receptors like those seen during phagocytosis of IgG-coated erythrocytes (35) and may be the manifestation of the modulation of Fc receptors from uninvolved membrane. A requirement for clathrin at the sites of ligand-receptor interaction may change the intracellular equilibrium from Golgi region to plasma membrane.

Although we have shown that clathrin coats are induced to assemble in increased numbers on the adherent plasma membrane of macrophages during frustrated phagocytosis mediated by receptor-ligand interaction, the exact mechanism and the role of this increased clathrin assembly have yet to be determined. However, frustrated phagocytosis provides a useful model for perturbing and analyzing clathrin dynamics.

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